



(RESEARCH ARTICLE)



BIOCONTROL OF THREE FUNGAL DISEASES OF SUGARCANE IN GREENHOUSES USING STRAINS OF *PSEUDOMONAS FLUORESCENS* AND *BACILLUS SUBTILIS* ISOLATED FROM THE RHIZOSPHERE OF SUGARCANE IN CÔTE D'IVOIRE

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ABSTRACT

Sugar cane (*Saccharum officinarum* L.) is a key crop for the economy of Côte d'Ivoire. However, domestic production remains insufficient to meet domestic demand, resulting in a sugar deficit for the country. This low productivity is largely due to fungal diseases. It is against this backdrop that this study was conducted, the overall objective of which was to evaluate the efficacy of two bacterial supernatants, based on *Bacillus subtilis* and *Pseudomonas fluorescens*, against the diseases red blight, pokkah boeng and sugarcane charcoal in a greenhouse setting. To this end, 150 samples of apparently healthy sugarcane cutting organs were collected from sugarcane plantations in the Zuenoula and Borotoukoro sugar complexes for use in the nurseries. Antifungal tests were then carried out in greenhouses against *Fusarium verticillioides*, *Fusarium subglutinans* and *Schizophyllum commune* in the presence of *Pseudomonas fluorescens* and *Bacillus subtilis*. The results indicated that the strains of *Pseudomonas fluorescens* and *Bacillus subtilis* were able to significantly reduce the severity of the diseases observed, with reduction rates of 80.95 ± 2.3 per cent, 80.95 ± 2.3 per cent and 82.80 ± 5.2 per cent for charcoal, pokkah boeng and red blight, respectively. These results suggest that *Bacillus*

subtilis and *Pseudomonas fluorescens*, when tested under field conditions, could be used effectively as biocontrol agents against fungal diseases affecting sugarcane crops in Côte d'Ivoire.

Keywords: Sugar Cane, Fungal Disease, *Bacillus Subtilis*, *Pseudomonas Fluorescens*, Côte d'Ivoire

1 INTRODUCTION

Sugar cane (*Saccharum officinarum* L.) is a perennial plant of the Poaceae family. It is native to New Guinea and is cultivated for its stems, which contain a sweet sap. In 2020, global sugarcane production reached approximately 1.92 billion tonnes, accounting for 79 % of total global sugar production (FAO, 2023). This sector constitutes a strategic agro-industrial sector for the production of sugar, ethanol and energy co-products. Côte d'Ivoire is a medium-sized producer in Africa. In 2018, the country's annual sugarcane production was estimated at 1.9 million tonnes, ranking it 53rd globally and 16th in Africa (OECD/FAO, 2023). Within the country, sugarcane is mainly used for direct consumption or in the sugar industry. It is also used in the cosmetics and pharmaceutical sectors. Furthermore, it is the main source of economic activity for the population in the northern part of the country (Kouamé *et al.*, 2010). Despite the importance of sugarcane, Côte d'Ivoire continues to have a sugar deficit. This deficit is generally caused by diseases affecting sugarcane cultivation, including bacterial diseases such as leaf blight caused by *Xanthomonas albineans*, and fungal diseases such as smut, red mould and pokkah boeng. These fungal diseases result in losses estimated at between 10 and 30 per cent of the national yield (Yao *et al.*, 2020). Although there are no comprehensive official national figures, fungal diseases cause economic losses amounting to between 20 and 40 per cent of the national yield. Thus, based on an estimated national sugarcane production of around 330,000 tonnes, a 20 per cent loss equates to 66,000 tonnes, representing nearly 13 million USD in annual economic losses. (Kouamé *et al.*, 2010). These fungal diseases are characterised by symptoms such as leaf chlorosis and green mottling of varying severity. The most susceptible varieties can be literally stunted to the point of resembling tufts of grass. Sometimes the stems are covered with cankers and the internal tissues are necrotic. This deterioration of the stems leads to a reduction in their sugar content and losses in yield. These diseases have caused crop losses of up to 40 per cent in the Borotou-Koro and Zuénoula sugar complexes (Yao *et al.*, 2020). In order to combat these pathogens effectively, pesticides – particularly synthetic fungicides from the benzimidazole, triazole and strobilurin families – are primarily used. However, the overuse of these synthetic fungicides in sugarcane plantations could lead to the emergence and rapid development of resistant strains, which would result in a resurgence of diseases (Cawoy *et al.*, 2011). In view of these adverse consequences, it is important to find alternative solutions that will enable us to continue combating plant pathogens whilst reducing the use of chemicals and also protecting the environment. These may involve the rationalisation of agricultural practices, the use of resistant plant varieties and/or the use of biopesticides (Thakore, 2006). It is against this backdrop that this study was conducted, the overall objective of which was to evaluate the efficacy of two bacterial supernatants derived from *Bacillus subtilis* and *Pseudomonas fluorescens* against the diseases red slime, pokkah boeng and sugarcane anthrax in greenhouses.

2 MATERIALS

2.1 Plant material

The plant material consisted of samples of apparently healthy sugarcane cuttings. These sugarcane cuttings were used to carry out biocontrol tests on pathogens in a greenhouse. These samples were collected from sugarcane plantations belonging to sugarcane growers in the Zuenoula and Borotou-koro areas. Diseased samples were used for the isolation of pathogenic fungi. Healthy sugarcane plants were used to carry out pathogenicity and biocontrol tests on pathogens in the greenhouse.

2.2 Fungal and bacterial material

The microbial material consisted, on the one hand, of strains of *Bacillus subtilis* and *Pseudomonas fluorescens* isolated from the rhizosphere of sugarcane in Côte d'Ivoire. In addition, pathogenic fungal strains of *Fusarium verticillioides*, *Fusarium subglutinans* and *Schizophyllum commune*, which cause diseases in sugarcane in Côte d'Ivoire, were used. These fungal and bacterial strains were obtained from the Laboratory of Food Biotechnology and Microbiology at the Food Science and Technology Teaching and Research Unit (UFR-STA) of Nangui Abrogoua University (Abidjan, Côte d'Ivoire). These strains were identified from symptomatic parts of sugarcane in Côte d'Ivoire, in accordance with the work of Okoue *et al.* (2025).

3 METHODS

3.1 Sampling area

Samples of apparently healthy sugarcane cuttings were selected from plots in the two sugarcane production areas, namely the Zuenoula Integrated Agricultural Unit (UAI), situated between 7°30' and 7°40' north latitude, and between 6°50 and 6°15 west longitude, and the Borotou-Koro Integrated Agricultural Unit (UAI), situated between 8°31 north latitude and 7°17 west longitude.

3.2 Sampling

Sugar cane cutting samples were taken from five (05) plots selected at random in each complex, making a total of ten (10) plots. Consequently, 150 samples of apparently healthy sugar cane cuttings were collected, at a rate of 75 samples per complex.

3.3 Evaluation of the antifungal activity of bacterial biocontrol agents against sugarcane pathogenic fungi in a greenhouse

3.3.1 Preparation of the fungal inoculum

Ten millilitres (20 mL) of sterilised distilled water were added to 14-day-old mycelial cultures of the pathogenic fungi. The surface of these cultures was scraped using a sterilised metal spatula. The resulting solution was homogenised in a test tube using a vortex mixer. 200 µL of this suspension was used to fill the wells of a Malassez cell in order to estimate the spore count. This number was adjusted to 10^8 spores/ml. 1 mL of a 1% glucose-agar solution was added to the spore suspension prior to inoculation. The purpose of this 1% agar solution is to facilitate the adhesion of the spores to the leaves (Silué *et al.*, 2018).

3.3.2 Preparation of the bacterial biocontrol agents suspension

A pre-culture of the biocontrol agents was carried out by inoculating 25 mL of YPG broth with 24-hour-old colonies and incubating for 8 hours at 30 °C. The colonies obtained from the pre-culture were used to inoculate two (02) 500 mL YPG broths, which were then incubated at 30 °C for 48 h. After 48 hours of incubation, the culture was centrifuged at 7,000 rpm for 10 minutes at 4 °C (Touré *et al.*, 2004). The supernatant was then collected and stored at 4 °C for biocontrol tests on plant pathogens in the greenhouse.

3.3.3 Inoculation and treatment of seedlings with bacterial biocontrol agent formulations

Thirty-six (36) two-month-old sugarcane seedlings were used in the experiment, with nine (9) seedlings per treatment. The treatments comprised seedlings not inoculated with the spore suspension and not treated with the bacterial supernatant (T0), control seedlings inoculated with the spore suspension but not treated (T1), seedlings inoculated with the spore suspension and treated with the supernatant of *Pseudomonas fluorescens* (T2), and seedlings inoculated with the spore suspension and treated with the supernatant of *Bacillus* sp. (T3). The midribs of the leaves and the stems of the sugarcane seedlings were inoculated with 1 ml of pathogenic fungal inoculum. Six (6) leaves were inoculated per sugarcane seedling, amounting to 6 ml of fungal inoculum per seedling. A total of 162 ml of fungal inoculum was used for thirty-six (36) sugarcane seedlings. Twenty-four (24) hours after inoculation, the sugarcane seedlings were sprayed separately with the bacterial supernatants, at a rate of nine (9) inoculated seedlings per supernatant. After treatment, the seedlings were placed in a greenhouse. The experiment was repeated three (3) times. The greenhouse was regularly humidified (twice a day) by spraying with a hand-held watering can to maintain a high humidity level (95–100 per cent). The purpose of humidification was to facilitate the germination of the fungal pathogen's spores. The progression of disease symptoms was monitored regularly over a period of three (3) months (Silué *et al.*, 2018).

3.3.4 Assessment of treatment efficacy

The progression of disease symptoms was monitored after each treatment. The ability of the two bacterial supernatants to protect against or reduce symptoms caused by fungal pathogens was assessed. This assessment was carried out by determining the infection rate, the disease severity index and the disease reduction rate.

3.3.4.1 Assessment of disease infection rates

The infection rate of the plants was assessed as the ratio of the number of leaves exhibiting disease symptoms to the total number of inoculated leaves (Issa *et al.*, 2017) according to the following formula (1):

$$Ti (\%) = \frac{n}{N} \times 100 \quad (1)$$

- $Ti (\%)$ = Disease infection rate
- n = Number of inoculated organs showing symptoms of the disease after treatment
- N = Total number of inoculated and treated leaves

3.3.4.2 Assessment of disease severity

Disease severity was determined using a visual rating scale ranging from 0 to 9 (Groth *et al.*, 1999; Cardoso *et al.*, 2004), where: 0 = No symptoms; 1 = 1–5 per cent; 3 = 6–10 per cent; 5 = 11–25 per cent; 7 = 26–50 per cent; 9 > 50 per cent of the leaf area infected. The disease severity index was determined for each treatment using formula (2) from Kranz (1988).

$$Is (\%) = \left(\frac{\sum (xi \times ni)}{NZ} \right) \times 100 \quad (2)$$

- Is = severity index (%)
- Xi = disease score
- ni = number of leaves with score xi
- N = total number of organs inoculated and then treated with bacterial supernatants
- Z = highest score

3.3.4.3 Assessment of the disease reduction rate

The disease reduction rate was determined by the ratio of the difference between the severity index of control plants infected with the pathogen and not treated with the bacterial supernatant and the the severity index of plants infected with the pathogen and treated with the bacterial supernatant, divided by the severity index of control plants infected with the pathogen but not treated with the bacterial supernatant, multiplied by 100 (formula (3)).

$$Tr (\%) = \frac{Io - I}{Io} \times 100 \quad (3)$$

- $Tr (\%)$ = Reduction rate
- Io = Severity index of control plants infected with the pathogen and not treated with the biopesticide;
- I = Severity index of plants infected with the pathogen and treated with the biopesticide

3.4 Statistical analyses

The data collected were analysed using Statistica software, version 7.1. A two-way analysis of variance (ANOVA II) was used to identify the antagonistic bacteria and the concentrations at which they significantly reduce or completely inhibit the in vivo growth of pathogens, as well as the development of disease symptoms. Where a significant difference was found, the means were compared using the Newman-Keul test at a 5 per cent significance level. The significance of the test is determined by comparing the probability (P) associated with the test statistic to the value $\alpha = 0.05$. Thus, when $P \geq 0.05$, it is concluded that there is no difference between the means. However, when $P < 0.05$, there is a significant difference between the means.

4 RESULTS

4.1 Effect of bacterial biocontrol agents on red blight

4.1.1 Development of red blight symptoms on sugarcane seedlings

One month after inoculation with fungal spore suspensions, neither the uninoculated and untreated sugarcane seedlings nor those inoculated and treated with bacterial supernatants showed any symptoms of red blight (Figure 1 A, C and D). Those inoculated with *Fusarium verticillioides* spores and left untreated exhibited the typical symptoms of red blight (Figure 1 B). The seedlings inoculated and treated with the supernatants of *P. fluorescens* and *Bacillus subtilis*, on the

other hand, developed no symptoms on their leaves. Normal plant development was also observed, with the emergence of new leaves and a deepening of the green colouration of the leaves (Figure 1 C and D).

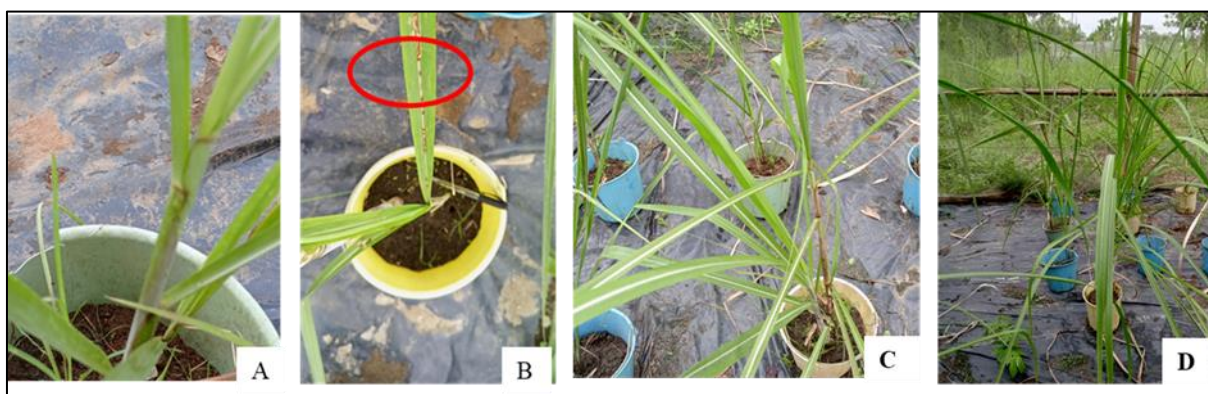


Figure 1: Symptoms of red blight observed one month after treatment with bacterial supernatants

- A – Seedling not infected with the *Fusarium verticillioides* spore suspension, showing no symptoms of red blight on the veins
- B – Seedling infected with a *Fusarium verticillioides* spore suspension and left untreated, showing symptoms of red blight on the veins
- C – Seedling infected with a *Fusarium verticillioides* spore suspension and treated with the supernatant of *Bacillus sp.*, showing no symptoms of red blight on the veins
- D- Seedling infected with a *Fusarium verticillioides* spore suspension and treated with the supernatant of *P. fluorescens*, showing no symptoms of red blight on the veins

4.1.2 Infection rates in seedlings treated for red blight

Three months after treatment, a 100 per cent infection rate was observed in seedlings inoculated with a suspension of *Fusarium verticillioides* spores and not treated with bacterial supernatants. Seedlings inoculated with the fungal spore suspension and treated with bacterial supernatants recorded infection rates of 50% and 65% respectively for the genera *Pseudomonas fluorescens* and *Bacillus subtilis*. Statistical analyses revealed a significant difference between the infection rates of untreated seedlings and those treated with bacterial supernatants at the 5 per cent significance level (Figure 2).

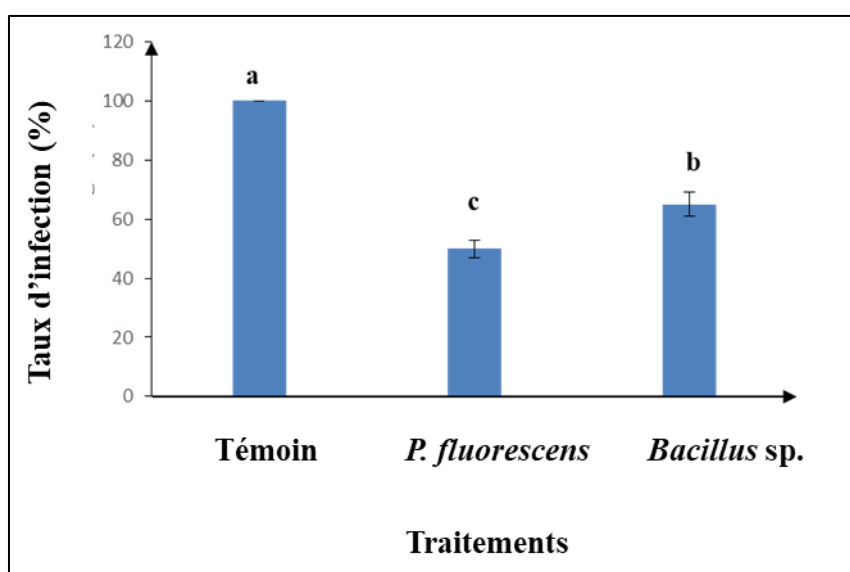


Figure 2: Infection rates of young sugarcane plants with red leaf spot disease in a greenhouse three months after treatment

Bars marked with the same letters are statistically indistinguishable at the 5 per cent significance level

4.1.3 Average severity index of red blight according to the treatments applied to the seedlings

An average severity index of 38 per cent was observed for the inoculated, untreated control seedlings. This severity is higher than that observed in seedlings treated with bacterial supernatants. Seedlings treated with the supernatant of *Pseudomonas fluorescens* showed a severity index of 9.33 per cent, whilst those treated with the supernatant of *Bacillus subtilis* showed an average severity of 15.33 per cent. The seedlings treated with the supernatant of *Pseudomonas fluorescens* thus exhibited a lower severity compared with those treated with. Statistical analyses also revealed a significant difference between these two values (Figure 3).

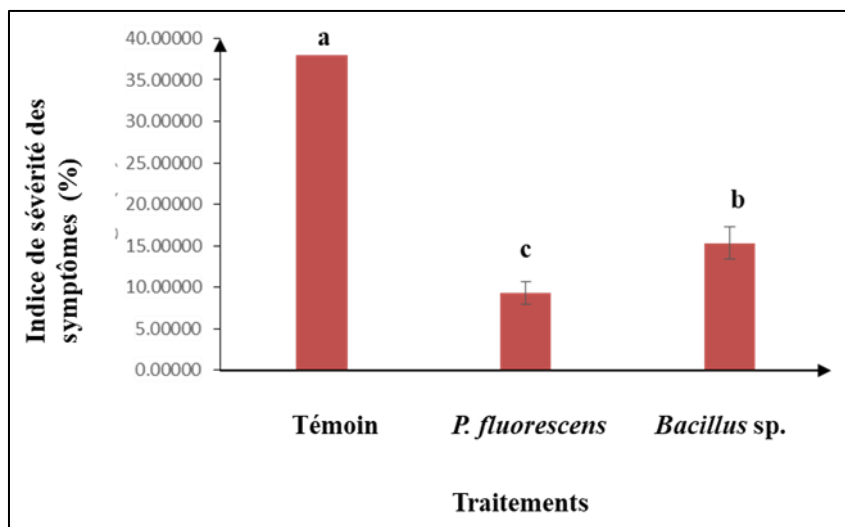


Figure 3: Average severity of red blight symptoms on young sugarcane plants three months after treatment

Bars marked with the same letters are statistically indistinguishable at the 5 per cent significance level

4.1.4 Reduction rates in the red glanders severity index following treatment

Average reduction rates of over 50 per cent were observed for both types of formulation. The reduction rates were 80.95% and 73.80% respectively for the supernatant of *Pseudomonas fluorescens* and that of *Bacillus subtilis*. These two values are statistically indistinguishable at the 5% significance level (Figure 4).

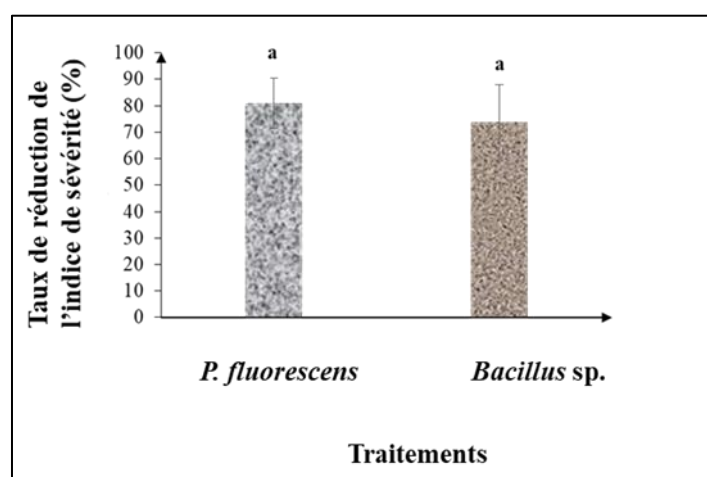


Figure 4: Average reduction in the severity index of red rot symptoms in young sugarcane plants three months after treatment

Bars marked with the same letters are statistically equivalent at the 5 per cent significance level

4.2 Effect of bacterial biocontrol agents on charcoal disease

4.2.1 Development of charcoal disease symptoms on sugarcane seedlings

One month after inoculation with fungal spore suspensions, the uninoculated and untreated sugarcane seedlings and those inoculated and treated with bacterial supernatants showed no symptoms of charcoal disease (Figure 5 A, C and D). Those inoculated with the *Schizophyllum commune* spore suspension and left untreated exhibited the typical symptoms of charcoal disease (Figure 5 B). The seedlings inoculated and treated with formulations of *P. fluorescens* and *Bacillus subtilis*, on the other hand, showed no symptoms on their leaves. Normal seedling development was also observed, with the emergence of new leaves and a deepening of the green colouration of the leaves (Figure 5 C and D).

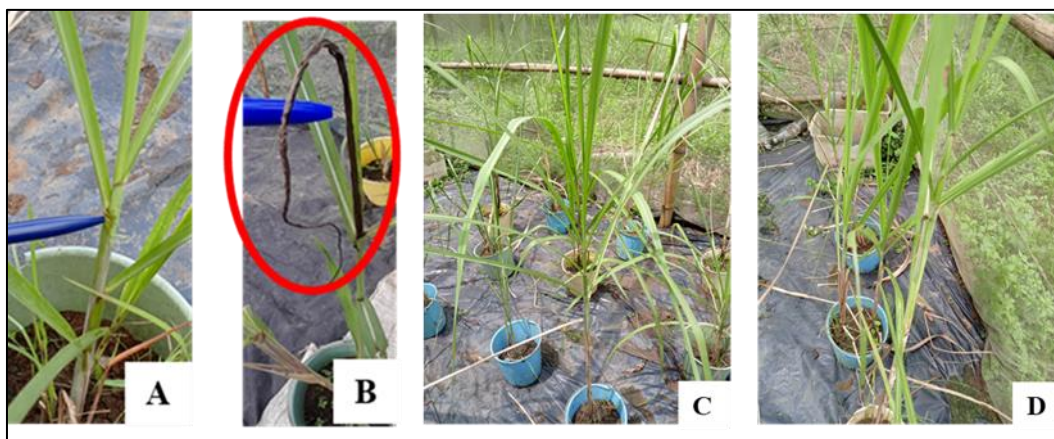


Figure 5: Symptoms of charcoal disease on the stems of sugarcane seedlings three months after treatment

- A – Sugarcane seedlings not infected with the *Schizophyllum commune* spore suspension and not treated with the bacterial supernatants, showing no symptoms of charcoal disease on the sugarcane stems
- B – Sugar cane seedling infected with a *Schizophyllum commune* spore suspension and not treated with bacterial supernatants, showing symptoms of charcoal rot on the sugar cane stem
- C- Seedling infected with a *Schizophyllum commune* spore suspension and treated with the supernatant of *Bacillus subtilis*. showing no symptoms of charcoal disease on the sugarcane stem
- D- Seedling infected with a *Schizophyllum commune* spore suspension and treated with the supernatant of *P. fluorescens*, showing no symptoms of charcoal rot on the sugarcane stem

4.2.2 Infection rates in seedlings treated for charcoal disease

Three months after treatment, a 100 per cent infection rate was observed in seedlings inoculated with the *Schizophyllum commune* spore suspension and not treated with the bacterial supernatants. Seedlings inoculated with the fungal spore suspension and treated with bacterial supernatants recorded infection rates of 70% and 75% respectively for the genera *Pseudomonas fluorescens* and *Bacillus subtilis*. Statistical analyses revealed no significant differences between the infection rates of seedlings treated with the *Pseudomonas fluorescens* supernatant and those treated with the *Bacillus subtilis* supernatant. However, statistical analyses showed a significant difference between the infection rates of untreated seedlings and those treated with the bacterial supernatants at the 5 per cent significance level (Figure 6).

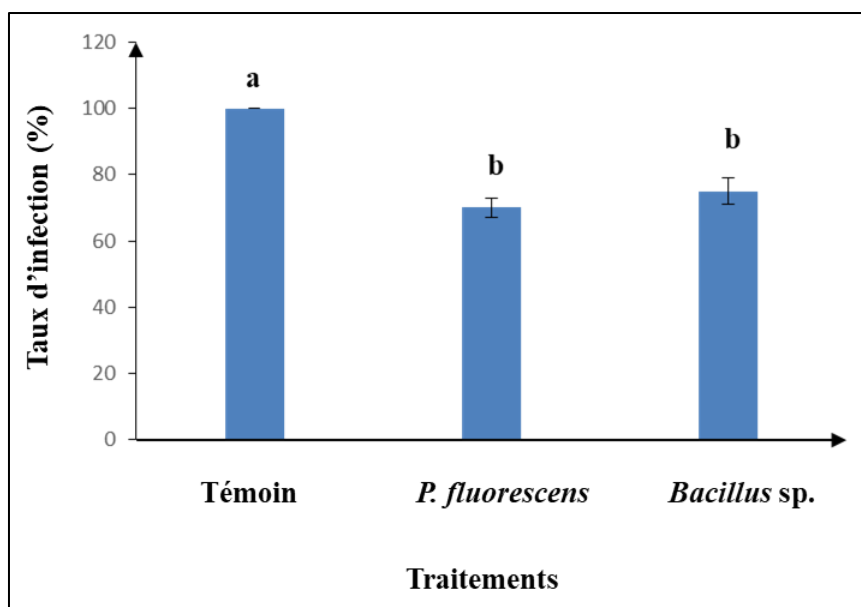


Figure 6: Infection rates of young sugarcane plants with charcoal disease in a greenhouse three months after treatment

Plots marked with the same letters are statistically indistinguishable at the 5 per cent significance level

4.2.3 Average severity index of charcoal disease according to treatments applied to sugarcane seedlings

An average severity index of 30.66 per cent was observed for the inoculated, untreated control seedlings. This severity was higher than that of the seedlings treated with the supernatants. Seedlings treated with the supernatant of *Pseudomonas fluorescens* showed a severity index of 10.33 per cent, whilst those treated with the supernatant of *Bacillus subtilis* showed an average severity of 7.33 per cent. The seedlings treated with the *Pseudomonas fluorescens* supernatant thus exhibited lower severity compared with those treated with the *Bacillus subtilis* supernatant. Statistical analyses also revealed a significant difference between these two values (Figure 7).

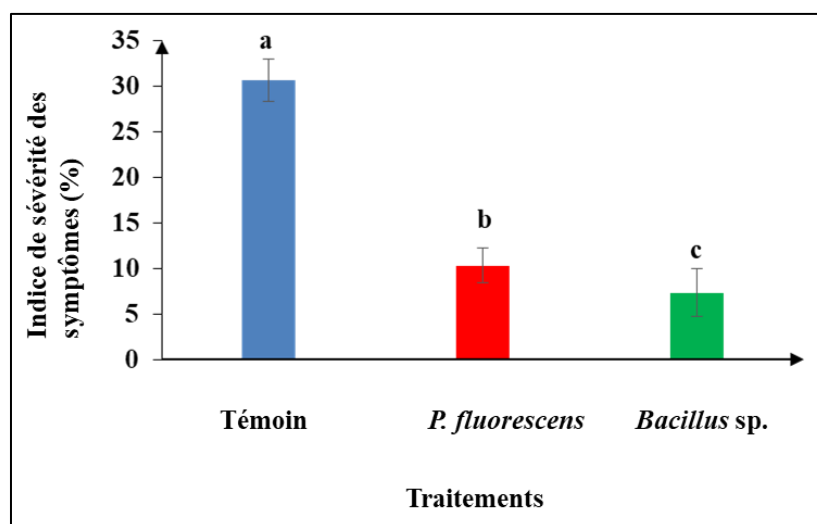


Figure 7: Average severity index of charcoal disease on young sugarcane plants three months after treatment

Plots with the same letters are statistically identical at the 5 per cent significance level, and those with different letters are statistically different at the 5 per cent significance level.

4.2.4 Reduction rates in the anthrax severity index following treatment

Average reduction rates in the anthrax symptom severity index of over 50 per cent were observed for both types of bacterial supernatants. The reduction rates were 65.19% and 82.19% respectively for the *Pseudomonas fluorescens*

based formulation and the *Bacillus subtilis*-based formulation. These two values are statistically different at the 5% significance level (Figure 8).

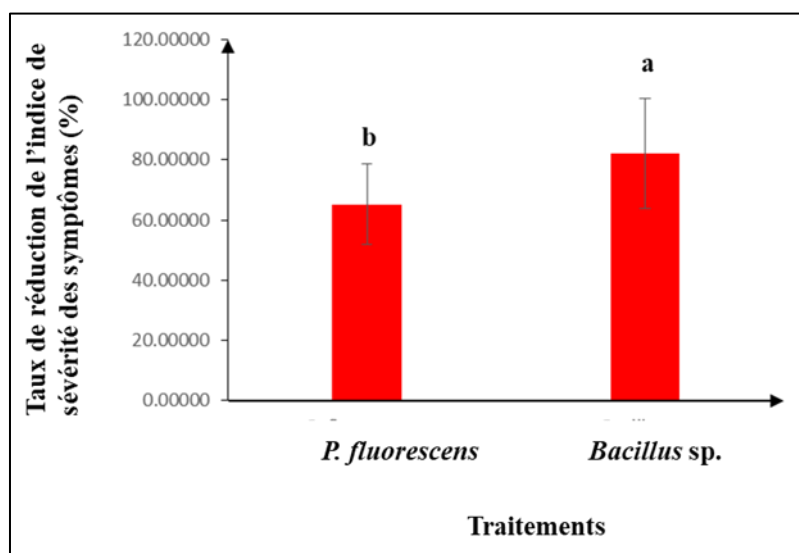


Figure 8: Average reduction in the anthracnose symptom severity index on young sugarcane plants three months after treatment in a greenhouse

Bars marked with the same letters are statistically equivalent at the 5 per cent significance level

4.3 Effect of bacterial biocontrol agents on 'Pokkah Boeng' disease

4.3.1 Development of symptoms of 'Pokkah Boeng' disease on sugarcane seedlings

One month after inoculation with fungal spore suspensions, the uninoculated and untreated sugarcane seedlings and those inoculated and treated with bacterial supernatants showed no symptoms of 'Pokkah Boeng' disease (Figure 9 A, C and D). Those inoculated with the *Fusarium subglutinans* spore suspension and left untreated exhibited the typical symptoms of 'Pokkah Boeng' disease, characterised by stem distortion with external particles and internal lesions resembling cuts (Figure 9 B). The inoculated and treated seedlings, on the other hand, showed no symptoms of 'Pokkah Boeng' disease on the stem. Normal development of these seedlings was observed, with the emergence of new leaves and a deepening of the green colouration of the leaves (Figure 9 C and D).

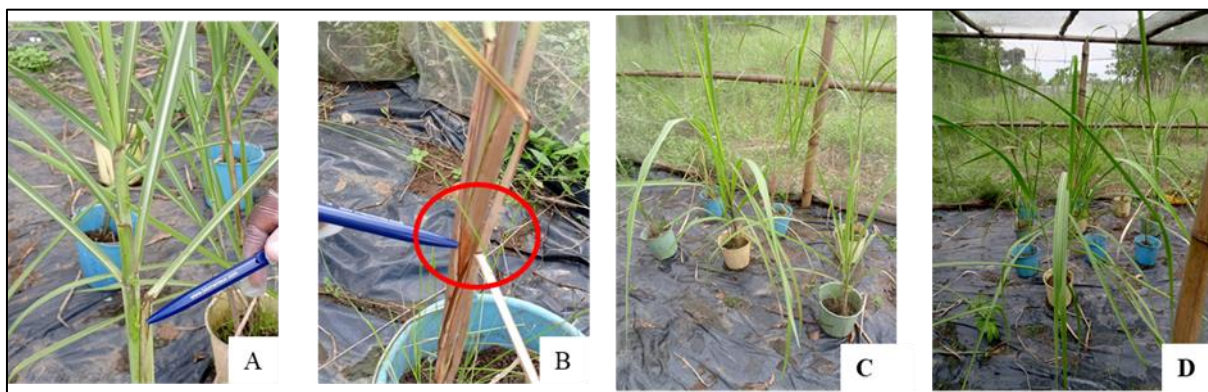


Figure 9: Symptoms of 'Pokkah Boeng' disease on the stems of sugarcane seedlings one month after treatment

- A – Uninfected sugarcane seedling treated with a *Fusarium subglutinans* spore suspension, showing no symptoms of 'Pokkah Boeng' disease on the sugarcane stem
- B – Sugar cane seedling infected with a *Fusarium subglutinans* spore suspension and not treated with bacterial supernatants, showing symptoms of 'Pokkah Boeng' disease on the sugar cane stem
- C- Seedling infected with a *Fusarium subglutinans* spore suspension and treated with the supernatant of *Bacillus subtilis*, showing no symptoms of 'Pokkah Boeng' disease on the sugarcane stalk
- D- Seedling infected with a *Fusarium subglutinans* spore suspension and treated with the supernatant of *P. fluorescens*, showing no symptoms of 'Pokkah Boeng' disease on the sugarcane stalk

4.3.2 Infection rates in seedlings treated for pokkah boeng disease

Three months after treatment, an infection rate of 95 per cent was observed in seedlings inoculated with a suspension of *Fusarium subglutinans* spores and not treated with bacterial supernatants. Seedlings inoculated with the fungal spore suspension and treated with the bacterial supernatants recorded infection rates of 65.44% and 65.42% respectively for the genera *Pseudomonas fluorescens* and *Bacillus subtilis*. Statistical analyses revealed no significant difference between these two values. However, these values are statistically different from those of the untreated control seedlings (Figure 10).

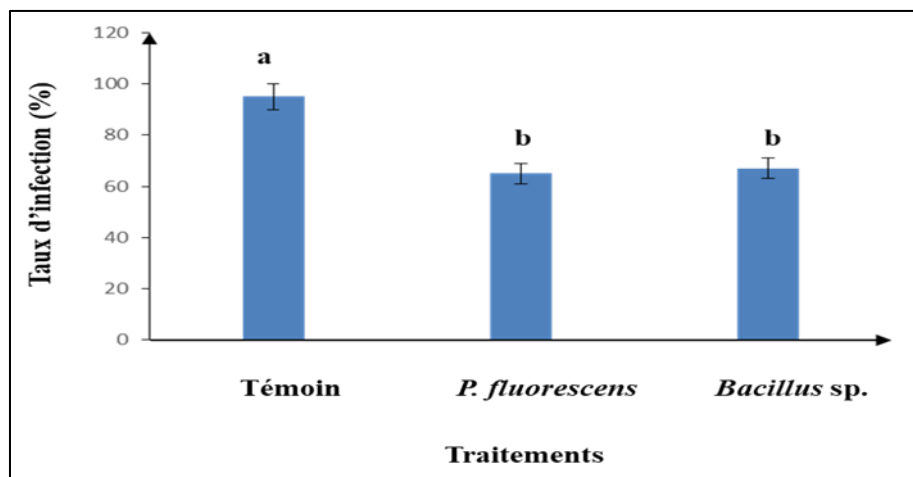


Figure 10: Infection rate of 'Pokkah Boeng' disease in young sugarcane seedlings in a greenhouse three months after treatment

Plots marked with the same letters are statistically equivalent at the 5 per cent significance level

4.3.3 Average severity index of pokkah boeng according to the treatments applied to the seedlings

An average severity index of 35.33 per cent was observed for the inoculated, untreated control seedlings. This severity was higher than that of the seedlings treated with the supernatants. Sugar cane seedlings treated with the supernatant of *Pseudomonas fluorescens* showed a severity index of 9.66 per cent, whilst those treated with the supernatant of *Bacillus subtilis* showed an average severity of 9.66 per cent. The seedlings treated with the supernatant of *Pseudomonas fluorescens* thus exhibited lower severity compared with those treated with *Bacillus subtilis*. Furthermore, statistical analyses did not reveal a significant difference between these two values (Figure 11).

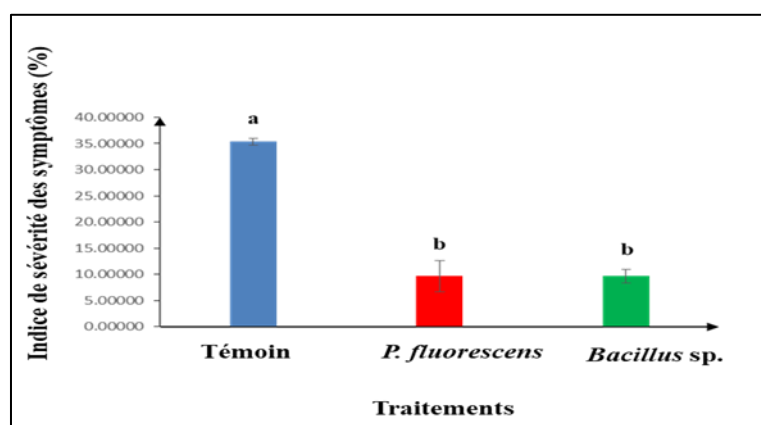


Figure 11: Average reduction in the severity index of pokkah boeng disease symptoms on young sugarcane plants three months after treatment

Bars marked with the same letters are statistically equivalent at the 5 per cent significance level

5 DISCUSSION

The overall aim of this chapter was to demonstrate the effect of bacterial biocontrol agent supernatants on pokkah boeng, red blight and sugarcane charcoal. The bacterial supernatants demonstrated their ability to significantly reduce the severity index of three sugarcane diseases in a greenhouse. This reduction in the severity index of the symptoms of the three diseases by the two bacterial extracts could be explained by the ability of these bacteria to produce substances that induce systemic resistance in the sugarcane against red blight, pokkah boeng and charcoal rot. Indeed, the isolates of *Bacillus subtilis* and *Pseudomonas fluorescens* are thought to possess two or three lipopeptide genes (srfAA, fenD and ituC), which are believed to be a major asset in promoting plant growth (Fatima *et al.*, 2009). These strains could play an antibacterial, antifungal and antiviral role, as well as acting as inducers of systemic plant resistance (Stanković *et al.*, 2012). Thus, several species of *Bacillus* sp. isolated from the plant environment possess the srfAA, fenD and ituC genes (Mora *et al.*, 2011). Furthermore, studies by Cawoy *et al.* (2015) revealed that strains of *B. subtilis* 98S possessed all three genes involved in the biosynthesis of surfactin, fengycin and iturin. However, *B. subtilis* 2504, used by Ongena *et al.* (2007) in their study possessed only the fengycin biosynthesis gene. Similar results were observed by Koua *et al.* (2020), who demonstrated that the *Bacillus subtilis* strains BL 1, BK 1, BL 2, BT 2, BT 5 and BT 10 were capable of inducing systemic resistance in cocoa plants against the cocoa swollen shoot virus (CSSV). They also demonstrated that these strains offer good potential for biological control against the swollen shoot virus and phytopathogenic moulds affecting cocoa, and that the induction of systemic resistance in cocoa seedlings by *B. subtilis* isolates reduced the severity of cocoa diseases caused by *Phytophthora palmivora*, *Lasioidiplodia theobromae* and CSSV. Similarly, Akram's 2008 research demonstrated the induction of systemic resistance in tomatoes and cucumbers by strains of *Bacillus subtilis* BTP1 and *Pseudomonas fluorescens* against grey mould caused by *Botrytis cinerea*. These results show that the bacterial strains used in these formulations appear to offer good potential for biological control of phytopathogenic fungi in sugarcane. This significant reduction rate and the strong growth of the plants compared with the control groups are thought to be linked to their ability to produce indole-3-acetic acid and to solubilise phosphorus. Indeed, Fatima *et al.* (2009) also demonstrated that high germination rates and vigorous plant growth are linked to the synthesis of indoleacetic acid (IAA). This ability of *Bacillus* sp. isolates to produce IAA is thought to have enhanced chlorophyll synthesis in the leaves (Fatima *et al.*, 2009). This would explain why the leaves of plants treated with the biopesticide formulations were greener than those treated with the phytopathogenic isolates. Similar results were observed with the *Bacillus subtilis* strains BTP1 and BC25 against *Botrytis cinerea* infecting tomatoes and cucumbers (Akram, 2008). The supernatant from a *Bacillus subtilis* isolate resulted in a considerable reduction in infection of cocoa pods by *P. palmivora* and *L. theobromae*. This demonstrates that the *Bacillus subtilis* supernatant contains antifungal substances such as fengycin and iturine, the genes for which were detected by PCR. Indeed, studies by Malfanova *et al.* (2012) determined that the supernatant of *B. subtilis* HC8 contained 65 µg/mL of iturine, 50 µg/mL of fengycin and 23 µg/mL of surfactin.

6 CONCLUSION

The aim of this chapter was to evaluate the efficacy of two bacterial supernatants derived from *Bacillus subtilis* and *Pseudomonas fluorescens* against red glanders, pokkah boeng and sugarcane anthrax in greenhouses. The various bacterial supernatants demonstrated their ability to reduce the severity index of red slime, 'Pokkah Boeng' and charcoal in greenhouses. These different biocontrol agents could serve as effective biopesticides in the biological control of sugarcane diseases in Côte d'Ivoire.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Disclosure of conflict of interest

The authors declare that they have no competing interests.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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